Cervical sprouting of corticospinal fibers after thoracic spinal cord injury accompanies shifts in evoked motor responses

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The adult central nervous system (CNS) of higher vertebrates displays a limited ability for self repair after traumatic injuries, leading to lasting functional deficits [1]. Small injuries can result in transient impairments, but the mechanisms of recovery are poorly understood [2]. At the cortical level, rearrangements of the sensory and motor representation maps often parallel recovery [3, 4]. In the sensory system, studies have shown that cortical and subcortical mechanisms contribute to map rearrangements [5, 6], but for the motor system the situation is less clear. Here we show that large-scale structural changes in the spared rostral part of the spinal cord occur simultaneously with shifts of a hind-limb motor cortex representation after traumatic spinal-cord injury. By intracortical microstimulation, we defined a cortical area that consistently and exclusively yielded hind-limb muscle responses in normal adult rats. Four weeks after a bilateral transsection of the corticospinal tract (CST) in the lower thoracic spinal cord, we again stimulated this cortical field and found forelimb, whisker, and trunk responses, thus demonstrating reorganization of the cortical motor representation. Anterograde tracing of corticospinal fibers originating from this former hind-limb area revealed that sprouting greatly increased the normally small number of collaterals that lead into the cervical spinal cord rostral to the lesion. We conclude that the corticospinal motor system has greater potential to adapt structurally to lesions than was previously believed and hypothesize that this spontaneous growth response is the basis for the observed motor representation rearrangements and contributes to functional recovery after incomplete lesions.

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Received: **2 November 2000**
Revised: **6 September 2001**
Accepted: **26 September 2001**

Published: **13 November 2001**

Current Biology 2001, 11:1766–1770

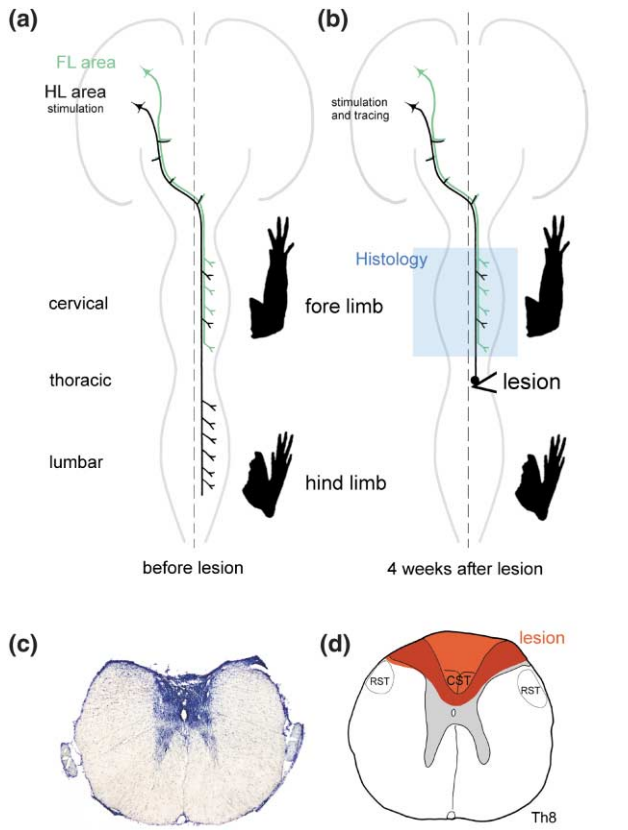
0960-9822/01/$ – see front matter

Results and discussion

Adult female Lewis rats were anesthetized, and their left motor cortex was exposed and stimulated by a tungsten microelectrode as described in the supplemental materials section, with the aim to identify an area that yields consistent and exclusive hind-limb (HL) responses. Consistent with previous studies [8, 9], this was found to be the case for stimulations at 2 mm caudal to bregma and 1.5–2.5 mm lateral to the midline. Stimulation at these coordinates usually activated hind-limb flexor muscles and, more rarely, extensor muscles. In two animals, muscles of the tail base were activated. We determined the stimulus threshold by increasing stimulus intensities and recording the lowest intensity at which a consistent movement was observed [7]. The average threshold was 49.6 μA (± 18.1 standard deviation [SD], n = 32), and animals with thresholds above 100 μA were not used for physiological evaluation. After the stimulation, the animals received an additional dose of anaesthetic (xylazine), and the scalp was sutured. Subsequently, the spinal cord was exposed at a mid-thoracic level (T8), and the dorsal columns, including the main component of the corticospinal tract (CST), were transsected bilaterally as described [10]. Care was taken not to compromise the dorsolaterally running rubrospinal tract (Figure 1). In a control group, the spinal cord was exposed but not transsected (sham operation). The tissue overlying the spinal cord was sutured, and the animals were left to recover on a heat pad.

After a survival time of 4 weeks, the animals were reanesthetized, and the motor cortex was again exposed. Stimulation was repeated at exactly the same coordinates, and the muscle responses and stimulation thresholds were recorded. For the sham-operated group, we were able to elicit HL activation at thresholds similar to those found previously; in the group of animals that had sustained a spinal cord lesion 4 weeks earlier, we observed HL activation only rarely. Instead, we observed activation of various other muscles rostral to the spinal cord lesion. Such muscles included those of the forelimb (FL), shoulder (SH), and whiskers (WH). Similar to the responses elicited before the lesion in the hind limbs, the vast majority of muscle contractions after the lesion in forelimbs occurred in flexor muscles. The thresholds required for eliciting

Figure 1



Experimental approach. **(a)** Before the spinal-cord lesion, the area of the motor cortex that consistently and exclusively yielded hind-limb muscle activation (HL area) was defined by intracortical microstimulation. Muscle responses were recorded by observation. **(b)** Two days and/or 4 weeks after a lesion of both corticospinal tracts at a mid-thoracic level, the stimulation was repeated in the same HL area, and muscle responses were recorded again. Subsequently, an anterograde axonal tracer was injected iontophoretically into the HL cortical area, and the innervation pattern of the axons originating from this area in the cervical spinal cord was studied and quantified ("Histology"). **(c,d)** The extent of the spinal lesions was controlled on cresyl violet-stained cross-sections through the lesion site. Both dorsal funiculi were transsected, which thus interrupted the ascending sensory tracts of the funiculi gracilis and cuneatus and the main corticospinal tract component. (d) Ventrally and laterally running propriospinal connections remained intact. Abbreviations are as follows: HL, hind limb; FL, forelimb; CST, corticospinal tract; and RST, rubrospinal tract.

Table 1

Changes in muscle activation after spinal injury upon microstimulation of the hind-limb motor cortex.

		After sham operation/injury	
Animals	Before injury	2 days	4 weeks
	Control group		
1	HLA (40)	n.d.	HLB (50)
2	HL (50)	n.d.	HL (70)
3	HL, TL (55)	n.d.	HL (60)
4	HL (50)	n.d.	HL (40)
5	HL (28)	n.d.	HL (50)
6	HL (40)	n.d.	HL (110)
7	HL (25)	n.d.	HL (30)
	Injury group		
1	HL (60)	n.d.	WH (60)
2	HL (80)	n.d.	FL, TR (55)
3	HL (60)	n.d.	WH (20), HL (80)
4	HL (70)	n.d.	SH, FL, HL (50)
5	HL (40)	n.d.	TR, FL (40)
6	HL (55)	n.d.	WH (90)
7	HL (20)	n.d.	SH, WH (40)
8	HL (25)	no response	FL (60), TR, TL (70)
9	HL (55)	no response	FL (60)
10	HL (40)	no response	n.d.
11	HL (40)	no response	n.d.
12	HL (60)	no response	FL (80)
13	HL (40)	no response	FL (30)
14	HL (40)	no response	no response
15	no response	no response	FL (30)
16	HL (60)	no response	FL (30)
17	HL (60)	no response	WH (60)
18	HL (55)	no response	no response
19	HL (30)	no response	FL (90)

Abreviations are as follows: HL, hind limb; FL, forelimb; WH, whiskers; SH, shoulder, TR, trunk; TL, tail; n.d., not done. Numbers in brackets indicate minimum stimulation threshold in μA.

responses in these muscle groups were similar to those in the HL muscles before the lesion and in the control group (57 μA ± 20.8, n = 15; Table 1). Thus, a significant shift in the cortical motor representation had occurred in response to the thoracic spinal lesion. Interestingly, animals in the experimental group exhibited large variations with respect to the muscles that were recruited by the former HL motor cortex.

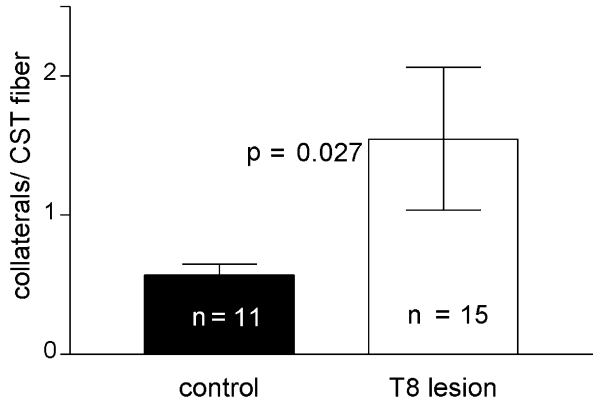
In a subset of animals in the injury group, we stimulated the HL area 2 days after introducing the lesion; however, we were never able to elicit any responses at this time point. Stimulation in a FL region at this time point resulted in FL activation at low thresholds (24.1 μA ± 5.8 SD, n = 11) in 11 out of 12 animals. We therefore concluded that the lack of response to HL area stimulation was due to the fact that the plastic processes resulting in FL or WH activation had not yet occurred and not to an effect of the recent injury (e.g., spinal shock). The level of the FL activation threshold was also very similar to that measured before the injury in the FL area (28.5 μA ± 6.7 SD, n = 10) and 4 weeks after the injury (29.4 μA ± 9.5 SD, n = 9). Thus, a general change in motor cortex excitability was not detected.

In some animals, the corticospinal axons of the stimulated area were traced anterogradely after the last physiological recording by stereotaxic, iontophoretic injection of biotin dextran amine into this area as described previously [11]. After a survival time of 2 weeks, the animals were killed, and their spinal cords were dissected. Cross-sections at

Figure 2

Innervation of the cervical spinal cord (approximate level C2–C3) by collaterals of corticospinal tract fibers originating from the HL area as observed on 50 μm thick horizontal sections. **(a)** In normal rats these CST axons (which branch extensively in the lumbar spinal cord, not shown) extend only few collaterals (arrowheads) into cervical spinal gray matter. **(b)** Four weeks after a mid-thoracic spinal cord lesion, the CST axons send many collaterals (arrowheads) into the cervical spinal cord, and dense innervation of intermediate laminae (arrows and insert) is observed. Some CST tract axons leave and reenter the plane of section and are therefore not depicted over their full length. The calibration bar represents 100 μm.

Figure 3



Quantitative analysis of the innervation of the cervical spinal-cord by corticospinal tract fibers originating from HL cortex. In the sham-lesioned group the average number of collaterals per HL CST fiber was consistently low. Four weeks after mid-thoracic spinal-cord lesion this number had significantly increased by almost 3-fold (Mann Whitney test). Similar to the observed shifts in the motor representation (Table 1), the variations in collateral numbers were large. n = number of animals.

level C1 and horizontal sections of the cervical segments C2–C6 were taken and processed for the presence of the tracer [11]. We took cross-sections of the lesion site and stained them by cresyl violet to control the correct extent of the lesion (Figure 1c,d). Sections were viewed in a brightfield microscope and were analyzed for (1) the number of CST fibers present in the dorsomedial funiculus of the C1 segment (averaged from three sections) and (2) the total number of collaterals emerging from the CST in segments C2–C6; all consecutive sections were counted (Figure 2). To correct for variations in tracing efficiency, we calculated an index from these two values, which yielded a measure for the collateralization of the traced HL CST in the cervical cord (collaterals per CST fiber).

In the experimental group that had received a spinal-cord lesion, we observed that the number of collaterals from CST axons formerly supplying the HL area in the lumbar spinal cord had increased greatly in response to the lesion (Figure 2). Many of these sprouts arborized in intermediate laminae of the gray matter and seemed to innervate spinal interneurons there (Figure 2, inset). Although in the control group 0.57 (± 0.08 standard error of the mean [SEM]) collaterals per CST fiber were seen to emerge from the HL CST in the cervical spinal cord, in the group of spinal cord-lesioned animals this number was increased by almost 3-fold to 1.55 (± 0.51 SEM, $p = 0.027$, Mann-Whitney test), indicating a strong sprouting response of these fibers into the cervical cord approximately 2.5 cm rostral to the injury (Figure 3). Interestingly, experimental animals that displayed a strongly increased HL CST innervation of the cervical spinal cord (animals 2, 4, and 5 of the injury group, with 2.2, 1.0, and 3.1 collaterals per HL CST fiber, respectively) also showed FL activation 4 weeks after injury. Further work will show whether shifts to, for example, whisker activation are accompanied by sprouting in brain stem areas. These studies will clarify whether strict correlations between changes in cortical motor representations and fiber sprouting can be drawn in all cases.

Rearrangements of the cortical motor and sensory maps in response to peripheral nervous system (PNS) and central nervous system (CNS) lesions have been well documented in rats [12, 13], primates [5], and human patients [14–18]. Several possible mechanisms underlying cortical map shifts have been discussed [4]. First, minor, normally subthreshold representations of movements or sensory inputs may become revealed at higher stimulation intensities after the main in- or output has disappeared. This, however, would require increased stimulation intensities

to demonstrate these functions. In our experiments, consistent with previously published studies [12], this was not the case. Stimulation thresholds did not differ significantly before and after spinal lesions. Second, it has been demonstrated that the unmasking of preexistent horizontal cortical connections, for example. through synaptic mechanisms such as long-term potentiation [19, 20] or reduced GABA-related inhibition [21, 22], can produce changes in cortical-representation areas. In both cases, subthreshold representations and the unmasking of preexisting connections, changes in cortical representations should already be apparent at early time points after the lesion, an effect we did not observe (Table 1). We therefore favor a third hypothesis, that structural changes involving fiber growth with changes in synaptic connectivity or formation of new synaptic connections may have occurred in response to the injury. This has recently been demonstrated for the sensory system [6, 23] and for unlesioned CST axons caudal to a lesion [24, 25]. In the present study, we observed a strong increase in the number of collaterals emerging from CST fibers that originated from a former HL area into the cervical spinal cord 4 weeks after a spinal lesion. This demonstrates that extensive sprouting can occur in the motor system.

It is unclear what the neuronal target cells of the newly formed sprouts are. Their terminations in intermediate laminae of the cervical cord suggest that they may be contacting local or long-descending interneurons. These possibilities are currently under investigation. The inductive molecular triggers by which fiber tracts are stimulated to sprout in order to compensate for lost function are unclear. Recent studies have yielded candidate molecules that were shown to induce collateral formation [26] or sprouting of lesioned CST axons during development or in the adult [27]. Similarly, a reduction of sensitivity to the tonic inhibition exerted by neurite growth inhibitors contained in the adult CNS [28–30] could explain the observed growth response. However, the role of these molecules in spontaneous compensatory sprouting of intact fiber systems after a lesion remains to be shown.

The interanimal variation in the experimental group was large, both for the observed shifts in motor representation and for the degree of increased HL CST collateralization, suggesting that different animals may adapt to injury by individual strategies, an observation supported by behavioral studies [31, 32]. In addition to the structural changes in the spinal cord described in this study and the previously reported adaptive synaptic plasticity in the motor cortex [33], brain stem mechanisms and other descending motor systems such as rubrospinal, bulbospinal, and propriospinal tracts may contribute to cortical map shifts, both by structural rearrangements and changes in synaptic strength. The observed reorganization of the cortical motor representations is therefore probably the result of functional and structural changes in several CNS systems. Recognizing the underlying mechanisms and understanding their molecular nature may eventually open avenues to enhancing functional recovery after CNS lesions and developing improved rehabilitation strategies for spinal cord and brain-injured patients.

Supplementary material

Supplementary materials and methods as well as an additional figure are available with this article on the internet at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgements

We thank Ms. Jeanette Scholl for excellent technical assistance and Dr. Marnie Halpern for critical comments on the manuscript. This work was supported by the Swiss National Science Foundation (grants 3100-045549.95/2 and 4038-043918.95/2 to M.E.S.) and the Spinal Cord Consortium of the Christopher Reeve Paralysis Foundation (Springfield, New Jersey, USA).

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